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Individualized Supplementation of Immunoactive Micronutrients and Severity of Upper Respiratory Infection Symptoms – a Randomized Intervention Study

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Article

Individualized Supplementation of Immunoactive Micronutrients and Severity of Upper Respiratory Infection Symptoms—A Randomized Intervention Study

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Abstract: Certain micronutrients exhibit immunomodulatory effects. However, no intervention has yet investigated the effect of an individualized supplementation on the severity of upper respiratory tract infections (URI). Therefore, we investigated whether a personalized supplementation moderates the incidence and severity of URI. Selenium, zinc, and vitamin D was measured from 59 healthy participants) in dried blood spots. Accordingly, a personalized supplement was provided with or without the respective micronutrients. We used WURSS-21 questionnaires to assess the disease status. The blood values converged during the intervention and micronutrients no longer differed at the end of the intervention period between treated and untreated volunteers. The incidence and severity of the illness did not significantly differ between the groups. However, when analyzing the WURSS-21 score by the intention to treat, the initially randomized treatment arm revealed a significantly higher score than the placebo arm. Upon acute administration, individualized combination of selenium, zinc and vitamin D does not reduce the number or contribute to a milder course of URIs. Therefore, supplementation in acute infectious situations seems questionable. Further studies must address the habitual diet in more detail to better understand the impact of individual micronutrient status on the prevention of URI.

Keywords: dried blood spots (DBS); upper respiratory tract infections (URI); personalized nutrition; vitamin D; selenium; zinc; WURSS-21

1. Introduction

A precisely tuned immune system is crucial to human health. It protects the organism against noxious factors, like pathogens and foreign substances. Nutrition is a major exogenous factor that modulates immune function [1]. To ensure the proper function of immune cells and physical barriers, the immune system needs multiple specific nutrients, which play synergistic roles at every stage of the immune response in humans [2,3]. Several vitamins (D, A, C, E, folate, B6, B12) and minerals (zinc, iron, copper and selenium) were, thus, documented to be essential for an optimal immune balance [4–6]. On the other hand, the mechanistic roles of micronutrients regarding the immune function have been well described recently. Some of the micronutrients like magnesium and Vitamin E have accepted European health claims with an immune-modulatory context [7].

Since the Corona pandemic, some micronutrients have been researched more extensively. In particular, vitamin D, selenium, and zinc have been discussed with regard to their effects on the immune system [8–13]. Vitamin D acts as an immune system modulator, preventing excessive expression of inflammatory cytokines and increasing macrophages' 'oxidative burst' potential. Additionally, it stimulates the production of antimicrobial peptides, which are crucial for protecting the respiratory tract from infections [14]. Observational studies have indicated a correlation between low blood concentrations of 25-hydroxyvitamin D3, the precursor of calcitriol, which is the active

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form of vitamin D, and viral respiratory tract infections, such as URI [14–16]. The association between zinc deficiency and immune dysfunction was established already 50 years ago, and since then, extensive research has been conducted in this field [17]. Zinc is involved in the regulation of the innate and adaptive immune response. It plays an essential role in lymphocyte formation, activation, and maturation, intercellular communication via cytokines, and the innate host defense [4,17,18]. Insufficient zinc levels can result in thymus atrophy, lymphopenia, and impaired cellular and antibody-mediated immune responses [17]. Selenium, which is also an essential micronutrient for human health, may contribute to a reduced immune function, some cancers, and viral diseases in case of a measurable deficiency [19]. Animal models and epidemiological studies have provided evidence that low selenium levels can facilitate genetic mutations and increase the virulence of specific viruses, such as coxsackievirus, poliovirus, and murine influenza [7].

Yet, the relationship between immunoactive micronutrient supplements and their effect on the occurrence and severity of respiratory infections remains inconclusive. Some studies suggest that standardized intake of micronutrients can reduce the occurrence of respiratory infections [8–14], while others report no significant effect [5,14–18]. Especially studies with older people and/or those with micronutrient deficiencies demonstrated that a micronutrient supplementation can reduce the incidence and severity of URI [11–13,20]. However, for younger, otherwise healthy individuals, these benefits have not yet been consistently shown [5]. Furthermore, no studies have explored the potential benefits of a personalized supplementation according to the individual micronutrient status.

Hence, the primary objective of our study was to investigate whether personalized supplementation based on individual blood levels of selenium, zinc, and vitamin D in healthy young volunteers could moderate the occurrence and severity of URI. To assess this, we used the Wisconsin Upper Respiratory Symptom Survey (WURSS-21), a standardized questionnaire for measuring URI symptoms [21,22].

2. Materials and Methods

Procedure for the intervention was followed in accordance with the ethical standards from the Helsinki Declaration and were approved by the ethics committee of the School of Medicine of the Technical University of Munich, Germany (75/21 S). All study participants provided online approval. The study was registered in the German Clinical Trials Register (DRKS): DRKS00023567.

2.1. Study Participants

For the intervention study cohort, in total, 94 healthy adults aged 18–65 years were recruited via announcements on social media (LinkedIn, facebook, Instagram) and local newspaper advertisements. Power calculation was performed using G-power 3.1, assuming a Cohen effect size of 0.8, alpha error of 0.05, power of 0.9, and a dropout rate of 10%. A total of 62 study participants were determined (28 per arm + 10%).

Participant enrollment started on April 23rd, 2021, and continued until November 23rd, 2021. The participants' eligibility was assessed with a detailed screening questionnaire. Exclusion criteria were age < 18 years, regular intake of supplements with vitamin D, selenium, zinc, and/or omega-3/omega-6 fatty acids, known intolerances to carrier substances of the supplement (guar gum, apple powder, potato starch), intention to go on vacation in southern climes (increased exposure to sunlight) and planned changes in dietary behavior (e.g. conversion to vegetarianism, veganism).

Randomization was performed online when participants registered for the study. Allocation to the study arms was carried out using Phyton Random Library. With a balancing feature to aim an equal group size. Figure 1 depicts the participant flow chart of the intervention study.

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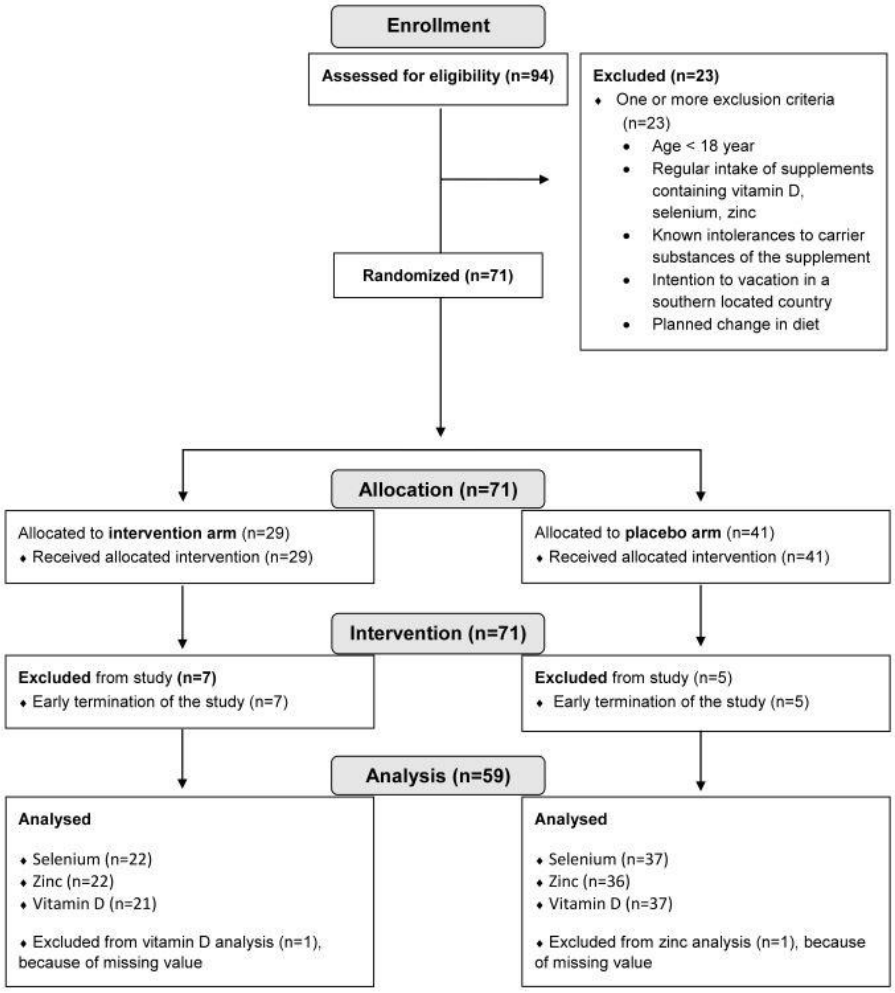

Figure 1. Intervention study participant flow diagram.

2.2. Study Design

This was a randomized, double-blind, placebo-controlled study. At the beginning of the study all participants were asked to collect capillary blood from their fingertips with a home test kit for dried blood spots (DBS) on their own. Based on the DBS analysis, participants in the treatment arm received micronutrient supplementation with individual amounts of selenium, zinc and/or vitamin D. Threshold blood levels for initiating supplementation were determined according to an algorithm from LOEWI® and were 0.14 µg/mL for selenium, 7 µg/mL for zinc and 95 nmol/L for vitamin D. Accordingly, participants randomized to the treatment groups received either one, two, three or no micronutrients. The placebo arm received only carrier material as a supplement. However, participants from the treatment arm not receiving any micronutrient in the supplement due to blood levels were subsequently also analyzed as receiving “placebo” compared to the "treatment" group who received either one, two, or all three micronutrients.

Figure 2 summarizes the study design, the originally randomized study arms and the spilt into individually supplemented groups. The treatment arm is colored two-tone, orange and orange-checkered. Full orange indicates supplementation with the respective micronutrient (treatment group), whereas volunteers appearing as orange checkered received no supplementation due to blood levels above the “critical supplementation level”. Consequently, we combined participants with an orange-checkered pattern from treatment arm with the volunteers from the placebo arm (grey-checkered) now referred as the placebo group (fully grey).

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The average time period between DBS analysis and start of supplementation was six weeks to allow production of the personalized supplement. The intervention period was 12 weeks. The Wisconsin Upper Respiratory Symptom Survey-21 (WURSS-21) questionnaire was filled out in every case of falling sick during the study phase. Every WURSS-21 questionnaire was filled out on seven consecutive days. At the end of the intervention period, the participants provided another DBS sample.

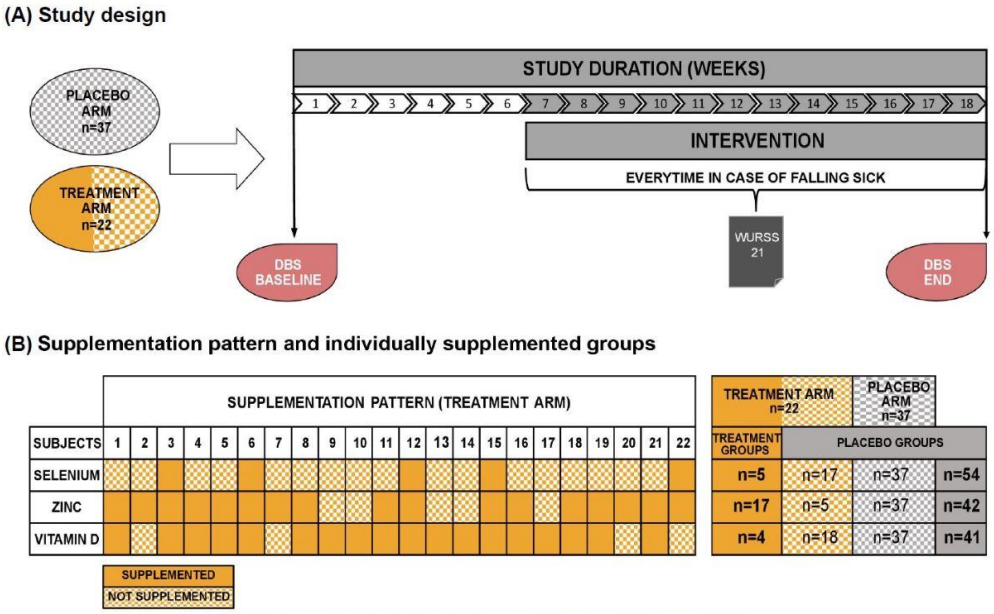

Figure 2. A: Study design of the intervention study. Dried blood spots (DBS) were sampled at the beginning and the end of the study. The whole study duration was approximately 18 weeks, of which the intervention lasted 12 weeks. WURSS-21 questionnaires were filled out in each case of falling sick. **B**: Supplementation pattern of the treatment arm: Full orange boxes indicate active treatment for the respective micronutrient; The orange-checkered boxes indicate no treatment and are, therefore, considered as receiving placebo for the respective micronutrient. Thus, each of the three placebo groups (selenium, zinc, vitamin D) appears as a sum of the placebo arm (grey-checkered) together with the untreated participants from the treatment arm (orange-checkered). "Arm" refers, therefore, to the original randomization whereas "groups" takes the real treatment into account.

2.3. Dried Blood Spot Analysis

After inclusion in the study, a home test kit with a detailed explanation for collecting DBS was send from the Core Facility Human Studies of the ZIEL- Institute for Food & Health, Freising, Germany to the participants. Participants independently collected DBS from their fingertips at the beginning of the study with a home test kit and after the intervention period. DBS were sent to Vitas AS, Norway for the analysis of selenium [µg/mL], zinc [µg/mL], vitamin D [nmol/L], iron [µg/mL], magnesium [µg/mL], omega-3 fatty acids and omega-6 fatty acids as % of fatty acid methyl ester (FAME). The personalized micronutrient supplement was produced according to the blood profiles of selenium, zinc, and vitamin D.

2.4. Study Product

The study product, provided by LOEWI®, was a powder consisting of carrier material guar gum, apple powder and potato starch. The product had to be taken daily during the complete intervention period (5 g). Whereas the placebo groups received the carrier material without micronutrients. The treatment groups received the powder with an individually calculated amount of selenium, zinc and vitamin D. Only nutrients below a defined threshold were added (see above). Figure 2B shows the supplementation pattern and the individually supplemented groups. Selenium was supplemented in a range of 51.0 µg to 97.5 µg (mean=66.9 µg), zinc from 7.2 µg to 26.7 µg

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(mean=4.5 µg) and vitamin D from 22.5 µg to 97.5 µg (mean=55.0 µg) per day. Accordingly, the amount of supplemented micronutrients did not exceed the official national recommendations.

2.5. The Wisconsin Upper Respiratory Symptom Survey

We used the WURSS-21 [21,22] and the four additional symptoms dry cough, fever, olfactory or taste disorder, and limb pain to evaluate the number of episodes and the severity of URI during the intervention period. For each case of illness during the supplementation, participants were asked to fill out a WURSS-21 for seven consecutive days. Symptoms were ranked from 0 (no symptom) to 7 (severe symptom). Daily WURSS summary scores were calculated by summing the scores (0-7) from each of the fourteen symptoms. The day with the highest WURSS score was used to evaluate disease severity ($WURSS_{max}$). An URI was defined from a $WURSS_{max}$ sum score of 7 or higher. The highest possible value for one day would be 105.3.

2.6. Data Analysis and Statistics

Data was analyzed in the R programming environment and GraphPad Prism 10. Results are presented as mean ± SD, unless otherwise indicated. *P*-values < 0.05 were regarded as statistically significant. Shapiro–Wilk test and quantil-quantil-plots were used to test normal distribution of data. According to distribution, either the t-test or Wilcoxon test was applied to assess differences in the cohorts. To assess differences within the placebo and treatment groups between baseline and the end of the intervention period the paired t-test was applied. One participant was excluded from the zinc and vitamin D analysis because of missing values due to low blood volume on the DBS filter paper.

3. Results

3.1. Participant Characteristics

In total, 94 volunteers were screened for our inclusion and exclusion criteria. Participant flow chart is shown in Figure 1. For analysis, the study cohort finally encompassed 59 participants with 37 subjects in the placebo arm and 22 subjects in the treatment arm. As shown in Figure 2B and Table 1, the individually supplemented groups for selenium, zinc and vitamin D were as follows: selenium (treatment group n=5, placebo group n=54), zinc (treatment group n=17, placebo group n=42), vitamin D (treatment group n=18, placebo group n=41). The placebo groups encompassed the placebo arm (randomized) plus the participants from the treatment arm without a deficit of the respective micronutrient. The treatment groups encompassed all participants with deficiencies for the respective micronutrient from the treatment arm. Table 1 depicts the study participants´ baseline characteristics and the division of the study arms into groups receiving the respective supplements.

Table 1. Baseline characteristics.

	Treatment arm	**Placebo arm**	**Overall**
n[1]	22	37	59
Sex (f/m), n	19/3	7/30	49/10
Age[2], years	26 ± 6	32 ± 13	30 ± 11
BMI, kg/m[2]	22.5 ± 3.8	22.8 ± 2.9	22.7 ±3.3
Individually supplemented groups[3]	**Treatment group**	**Placebo group (untreated treatment arm+ placebo arm)**	**Overall**
Selenium, n	5	17+37	59
Zinc, n	17	5+37	59
Vitamin D, n	18	4+37	59

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[1] n= Study participants finally included in the analysis of intervention and observation cohort. [2] Age and BMI represent mean ± SD. [3] Individually supplemented groups are the division into groups according to the blood values in respective micronutrient and refers to real treatment.

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3.2. Effect of Personalized Supplementation on the Micronutrient Status

In the following, volunteers in the intervention arm who did not receive any micronutrient treatment according to their baseline blood levels are analyzed together with the originally randomized placebo arm as one group. Figure 3 and Table 2 how the effect of individual supplementation with selenium, zinc and vitamin D. At baseline, the placebo and treatment groups significantly differed in terms of the status of all three micronutrients. As expected, the placebo group showed significantly higher levels than the treatment group (p-value$_{selenium}$=0.002, p-value$_{zinc}$=0.024, p-value$_{vitamin\ D}$ =0.017). After the supplementation period of 12 weeks, the differences between the placebo and treatment group were no longer significant (p-value$_{selenium}$=0.660, p-value$_{zinc}$=0.946, p-value$_{vitamin\ D}$=0.628). Thus, at the end of the study duration, the values of the different treatment groups adjusted to those of the untreated placebo groups (and Table 2). Although all blood levels of single micronutrients in the supplemented group seem to increase over time, only vitamin D reached statistical significance after 12 weeks compared to the baseline levels (p-value=0.017).

Table 2. Micronutrient status observed in DBS at baseline and after 12 weeks (absolute changes and in %).

	Baseline[1]	**End**	**Change (%)**
A: Selenium	**[µg/mL]**	**[µg/mL]**	
Placebo group (n=54)	0.171 ± 0.036	0.152 ± 0.046	-6.03 ± 36.95
Treatment group (n=5)	0.125 ± 0.009	0.147 ±0.028	17.65 ± 16.32
p-value[2]	0.002	0.660	0.048
B: Zinc	**[µg/mL]**	**[µg/mL]**	
Placebo group (n=42)	6.53 ± 1.86	6.05 ± 2.15	-3.48 ± 36.75
Treatment group (n=17)	5.34 ± 0.81	5.61 ± 1.16	6.90 ± 24.93
p-value	0.024	0.946	0.069
C: Vitamin D	**[nmol/L]**	**[nmol/L]**	
Placebo group (n=41)	93.89 ± 38.16	93.89 ± 30.59	15.16 ± 58.17
Treatment group (n=17)	70.46 ± 15.25	88.40 ± 27.61	31.45 ± 53.58
p-value	0.017	0.628	0.144

[1] Data is presented as mean ± SD. [2] P-value describes significance between individually supplemented treatment groups and placebo groups. *P*-values < 0.05 were regarded as statistically significant. Significance was calculated using a Wilcoxon test or a t-test, according to the data distribution.

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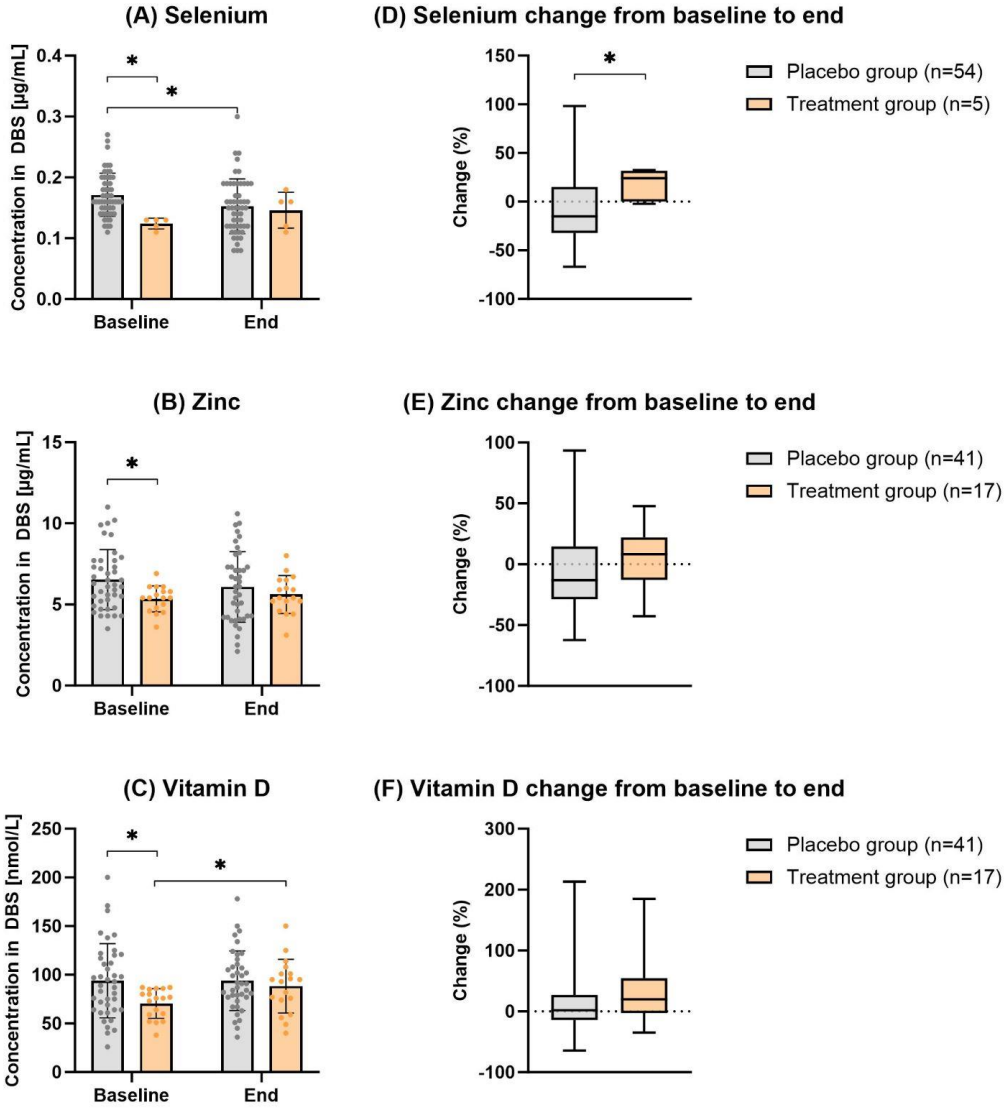

Figure 3. Micronutrient status between baseline and after 12 weeks of the intervention (A-C, left) and the corresponding changes in % (D-F, right). Comparison of the placebo and treatment group at baseline and at the end (according to distribution: t-test or Wilcoxon test). For changes of baseline and final values within the study group a paired t-test was used. P-values < 0.05 were regarded as statistically significant. Barplots with individual values (A-C), the bars represent the mean with SD. Boxplots (D-F) are in the style of Tukey, the line represents the median One participant was excluded from the zinc and vitamin D analysis because of missing values due to low blood volume on the DBS filter paper.

3.3. Individual Supplementation with Micronutrients does not Reduce Frequency or Moderate Severity of URI

During the entire study duration of seven months, a total of 16 URI occurred in the study cohort; seven in the placebo arm and nine in the treatment arm. A summary of URI cases in the individual supplemented groups is provided in the Table 3.

Interestingly, WURSS*max* did not significantly differ in the single supplemented treatment and placebo groups but, noteworthy, the treatment and placebo arms, analyzed according to the initial intention to treat, show significant differences in the severity score (p-value=0.045).

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Table 3. Participants falling sick (n=16) during study period.

		Placebo		Treatment		
		n of falling sick	WURSS$_{max}$[1]	n of falling sick	WURSS$_{max}$	*P*-value[2]
Study Arms		7	28.57 ± 11.84	9	43.11 ± 13.45	0.045
Individually supplemented groups	Selenium	14	36.71 ± 14.64	2	37.00 ± 18.38	ns
	Zinc	9	31.00 ± 12.70	7	44.14 ± 13.84	ns
	Vitamin D	8	33.37 ± 17.46	8	40.13 ± 10.72	ns

[1] Data is presented as mean ± SD. [2] *P*-values < 0.05 were regarded as statistically significant. Significance was calculated using a Wilcoxon test or a t-test, according to the data distribution.

4. Discussion

Despite various attempts in the past to supplement micronutrients as an adjuvant treatment for infections, the data remains controversial, and there is still limited evidence that individual micronutrients can be used to prevent or ameliorate URI symptoms [5,8,14–17]. Therefore, we aimed to investigate if a combination of selected immunoactive micronutrients (selenium, zinc, and vitamin D) with a personalized supplementation strategy based on individual blood levels could be effective to reduce the frequency and severity of URI. To our knowledge, this is the first study with such an approach.

First, we could see that supplementation over 12 weeks, in line with existing national recommendations [23–25], was able to alter relatively low blood levels of the selected micronutrients. The blood values became similar between the groups and the previously observed significance for selenium, zinc and vitamin D disappeared between the groups after 12 weeks. The fact that the blood levels after supplementation within the treatment group did not reach statistical significance between baseline and the end of the study may be due to a small number of individuals being effectively treated. This could be related to a selection bias, where participants are more likely to join the study if they have some interest in healthy eating and are less likely to be nutrient deficient due to a balanced diet compared to the average population. In addition, we used only moderate doses to correct deficiencies, which may require more time to be reflected in the results. It should also be noted that our intervention study was conducted in otherwise healthy individuals who did not have clinically relevant deficiencies for the three micronutrients according to EFSA reference values [23–25]. Furthermore, the blood levels at which our participants received the respective supplementation were interpreted by an algorithm from LOEWI®, which is a continuum, and, therefore, the subjects received individual doses that did not follow a yes-no approach related to fixed thresholds.

Although we achieved comparable levels at the end of the trial with the individually composed nutritional supplement compared to the control group with normal blood values, we did not see an improvement in the occurrence or severity of URI in the treated participants. Our results show that neither the occurrence nor the severity of URI during the study period differed between the intervention and placebo groups. The relatively low frequency of infection events is due, among other things, to the fact that the applicable contact restrictions of the Corona pandemic prevented more URIs during the study period. Interestingly, when comparing the overall treatment group with the placebo group and analyzing the severity of illness according to intention to treat, the treatment group had significantly higher WURSS$_{max}$ scores. This result may seem surprising at first glance, as there are several studies suggesting a beneficial effect of immunoactive micronutrient supplementation on URI and Covid-19, especially vitamin D [8,26,27] and zinc [10,14]. For URI, however, a positive effect was only shown in severe deficiency states [19,28]. Affirmingly, several other studies were also not able to show any significant amelioration in the frequency and severity of

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URIs when supplementing micronutrients [12,14,15,17,18]. For instance, a recent meta-analysis by Vlieg-Boerstra et al. found no association between the supplementation of proposed immunoactive nutrients, for example, vitamin D supplementation, and improved immune system response in the general European population [5]. Furthermore, the beneficial effects of supplementation reported in the literature have mainly been observed in studies of (older) individuals with low blood levels of micronutrients and in longer-term supplementation studies [11,12,28,29]. In our study, all our participants were younger and did not show signs of deficiency in the selected micronutrients, and it must be noted that we only used short-term supplementation without pharmacological doses. Regarding the higher severity scores of the participants in the treatment arm, it is of course possible that the small number of participants may have biased our results. However, the findings show that even with adequate micronutrient status and supplementation with immunomodulatory micronutrients, people are not resistant to URIs and that other factors may contribute to the incidence and severity of URIs.

5. Conclusions

In conclusion, our results suggest that a strong immune defense does not depend solely on acute supplementation with individual micronutrients or a combination of vitamin D, zinc and selenium. Rather, it appears to be a more complex interplay beyond the classical immunoactive micronutrients, pointing to the importance of a balanced diet providing all critical bioctives. Therefore, it is advisable to maintain a healthy, balanced diet that meets the recommended daily values of these nutrients to ensure optimal functioning of physical barriers and immune cells. In addition, we believe it is important to consider other factors such as age, sex, comorbidity and lifestyle. For future studies, we propose a more global approach that considers multiple factors, such as diet, when addressing immune system integrity

Author Contributions: Conceptualization, Melanie Haas, Laura Schinhammer and Thomas Skurk; Data curation, Laura Schinhammer; Funding acquisition, Thomas Skurk; Investigation, Melanie Haas and Laura Schinhammer; Project administration, Laura Schinhammer and Thomas Skurk; Resources, Thomas Skurk; Supervision, Thomas Skurk; Visualization, Melanie Haas; Writing – original draft, Melanie Haas; Writing – review & editing, Beate Brandl, Laura Schinhammer and Thomas Skurk.

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Institutional Review Board Statement: Procedure for the intervention was followed in accordance with the ethical standards from the Helsinki Declaration and were approved by the ethics committee of the School of Medicine of the Technical University of Munich, Germany (75/21 S, February 25, 2021). All study participants provided online approval. The study was registered in the Ger-man Clinical Trials Register (DRKS): DRKS00023567.

Informed Consent Statement: Written informed consent has been obtained from the patient(s) to publish this paper

Data Availability Statement: Data described in the manuscript will be made available upon request.

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Conflicts of Interest: The corresponding author declares that the other authors have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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